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LYMPHOCYTES AND PLASMACYTES IN NUCLEOPROTEIN METABOLISM

By

MARGARET A. KELSALL AND EDWARD D. CRABB

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LYMPHOCYTES AND PLASMACYTES IN NUCLEOPROTEIN METABOLISM*

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Lymphocytes and plasmacytes are enigmas because their ability to synthesize and store the nucleic and amino acids for use by other cells enables them to exhibit multiple and seemingly unrelated functions. The presence of lymphocytes and plasmacytes has been confusing because concentrations of these cells are normal in some organs, but are indicative of pathological conditions in others. Furthermore, aggregations of these cells normally occur in some tissues, such as the tunica propria of the intestinal mucosa, but the number fluctuates in other normal organs. In addition, there may be a heavy infiltration of one or both of these cells during one stage of an inflammatory process, but few if any in the same region during earlier or later stages of the same physiological or pathological process.

The literature on lymphocytes and plasmacytes is confusing and sometimes apparently contradictory because these cells have been studied by investigators working in many branches of the clinical and biological fields. Some authors barely mention, or altogether fail to consider, the presence of lymphocytes and plasmacytes, while others contend that the functions of the lymphocyte or plasmacyte pertain exclusively to their respective fields of research.

The purpose of this work is to consider the available information for and against the tenet that lymphocytes and plasmacytes are primarily true trephocytes (feeding or nutritive cells) that synthesize and store nucleoproteins. The small lymphocyte, which is a motile and mobile cell incapable of perpetuating itself, transports nucleoprotein to sites or regions where its components are used by other cells in growth and maintenance. Lymphocytes and plasmacytes also may furnish trephones (nutritive substances supplied by trephocytes) for pathological growth processes, which may be initiated by various agents, as well as for the growth that accompanies degenerative processes, such as fibrosis. The

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significance of lymphocytes and plasmacytes in growth processes is related chiefly to the fact that they are accessory cells that synthesize, store, and supply nucleoproteins in the most economical manner possible. Thus, the significance of a single lymphocyte is that it delivers a supply of nucleoproteins in balanced proportions and a packaged form, and that infiltrations of these cells deposit their proteins in a delimited area.

Lymphoid tissue, the parenchyma of which is composed primarily of small lymphocytes with varying numbers of plasmacytes, is advantageously located between certain "free" body fluids and the vascular system and has the function of synthesizing and storing nucleoproteins. Lymph nodes, with their afferent and efferent lymphatics, and the thymus, which lacks afferent lymphatics, represent nucleoprotein storage systems interposed between tissue fluid, chyme or chyle, and the blood vascular system. Intestinal lymphoid tissue and the lymphocytes and plasmacytes in the tunica propria of the gastrointestine serve as nucleoprotein storage systems located between the lumen of the gastrointestine and the hepatic portal vein and, also, indirectly between the lumen of the gastrointestine and the intestinal lymphoid tissue (via the central lacteals and other intestinal and mesenteric lymphatics, mesenteric lymph nodes, and the thoracic duct) and the systemic blood vascular system. The spleen, which is situated within the blood vascular system between peripheral arterial and portal venous blood, also has a function of nucleoprotein storage that contributes to the functions of the liver in maintaining plasma protein levels.

LYMPHOCYTES AS TREPHOCYTES

The idea that lymphocytes have a function in nourishing other cells is not new; it has been considered by several investigators. As early as 1716, Dionysius recorded the belief that the thymus secreted a fluid that emptied into the subclavian vein and furnished nutritive material for the newborn. This idea was supported by Verheyen (1706), Haler (1748), Mascagnius (1787), Lucae (1811), Becher (1826), and Haugstead (1832) in their descriptions of the lymph vessels of the thymus.[159] In 1873, His called attention to the apparent entry of leukocytes into vertebrate ovarian ova.[111]

The specific function of lymphocytes (including thymocytes) as storage cells for nucleoprotein was considered by Carrel.[28] Dustin[51-55] held that the thymus has a function in nuclear regulation, that formation and lysis of lymphocytes regulate the available nucleoproteins, that a reserve of nucleoproteins is necessary for all active cell division, and that lymphoid tissues and lymphocytes have a function in cytoregulation in adult animals. Dustin[51] credits Carrel and Ebeling[29] with establishing the fact that leukocytes carry trephones that are essential for maintenance and growth in tissue culture, and he[52] concludes that the function of thymocytes and lymphocytes[56] is to store and to liberate nucleoproteins.

Work in tissue culture has shown definitely that lymphocytes have growth-promoting properties.[28,29] Carrel and Ebeling[29] report that lymphocytes were able to maintain themselves and multiply in a medium containing only blood serum, while segregated fibroblasts and epithelial cells in this medium were not able to synthesize protoplasm. However, the addition of lymphocytes or of leukocytic extracts was followed by mitotic proliferation of the fibroblasts and epithelial cells. Jordan and Speidel[92] also believed that lymphocytes are embryonic relics that at times stimulate any region needing stimulation for growth. Liebman[111] describes lymphocytes entering the ovum of the newt and concludes that they act as trephocytes. However, he does not maintain that the lymphocyte is the only source of nutritives for the ovum, because the follicular cells are also a source.

The possibility that lymphocytes might provide energy, as well as growth-promoting substances, also has been regarded. In 1919 Bristol[22] considered the importance of lymphocytes in growth processes and noted that small lymphocytes were composed chiefly of nuclear material and that their chief function is the transportation of energy in nuclear enzymes for normal, proliferating cells. He also stated that concentrated numbers of lymphocytes may aid nutritionally and supply energy to the normal cells around areas of inflammation and injury. It is interesting to compare the prediction Bristol made in 1918 with a recent statement made by Ehrich and Seifter[61] in 1953. Bristol[21] suggested that "overstepping" of normal cell energy, or of acceleration in biochemical activities, resulted in accelerated energy production and growth. Ehrich and Seifter stated that lysis of lymphocytes and plasmacytes provides protein that may be used in gluconeogenesis and may supply nucleic acids and their split products to furnish energy and building stones for protein synthesis.

CHARACTERISTICS OF SMALL LYMPHOCYTES

The small lymphocyte has several structural, physiological, and chemical characteristics that are germane to its functioning as a trephocyte. The size of the small lymphocyte facilitates transportation of this cell in blood and lymph capillaries because it is about the same size as the erythrocytes in various species of laboratory mammals.[93] Several authors have reported that small lymphocytes of man are also the same size as the erythrocytes. Maximow and Bloom[123] state that small lymphocytes are 6 to 8 μ in diameter and that erythrocytes average 7.74 μ, while Lambert[103] states that small lymphocytes are 6 to 8 μ in diameter and are about the same size as the erythrocytes – 7.5 μ in man. Krafka[102] gives 7 to 10 μ for the diameters of human small lymphocytes and 7.5 for erythrocytes. In contrast, the larger size of the mature plasmacyte, which is approximately twice the size of the small lymphocyte, facilitates retention of nucleoproteins within the region in which the plasmacyte develops and provides a mechanism for the concentration and retention of proteins for local re-use by cells in a subsequent step of a process, such as wound healing.

The motility and mobility of the small lymphocyte increase its efficiency as a trephocyte, for it enables one type of cell to transport desoxyribonucleic acid (DNA) and histone. By infiltration, small lymphocytes readily concentrate nucleoproteins in tissues where they are needed because this cell occurs in blood, lymph, tissue fluid, and cerebrospinal fluid. The small lymphocyte can provide components of nucleoproteins for the liver, skin, or any other structure or organ, with the exception of certain parts of the central nervous system and ovary, which have special trephocytes.

There are several cytologically visible means by which small lymphocytes can contribute trephones to body fluids and to other cells. In addition to being able to release substances by dialysis and by cytolysis, processes that occur in many cells, small lymphocytes can release trephones by a process known as cytoplasmic budding[122] and by formation of long cytoplasmic tubes through which substances are ejected.[63] Englebert[63] describes the release of cytoplasmic fragments from lymphocytes in cultures of thymus of chick embryos. She states that only once has a tubule-bearing cell similar to a "changed lymphocyte" been reported in the literature. This was an unlabeled and undescribed illustration by Watney, in 1882, noted in fetal calf thymus. Williamson[175] also described the presence of nonnucleated cytoplasmic bodies in imprints of lymph nodes of rabbits, and he suggests that these occur from buds developing on lymphocytes. Furthermore, he states that these cytoplasmic bodies contain mitochondria. In addition, exchange of material between nucleus and cytoplasm is usually by diffusion of submicroscopic substances. For example, passage of ribonucleotides from the nucleus to the cytoplasm of cells has been thought to be effected by means of diffusion of partly hydrolyzed substances[50] not indicated by histochemical staining methods.[158] Follicular cells of Hemiptera have been reported by Schrader and Leuchtenberger[158] to furnish nuclear as well as cytoplasmic material to the growing egg by extrusion through the nuclear membrane. Droplets of DNA thus transported became Feulgen negative before reaching the egg. Possibly the interchange of DNA also may occur by means of the method suggested by Anderson:[1] by extension of DNA through the porous nuclear envelope when cells are in an acidic solution.

The absence of mitosis in the small lymphocyte is another characteristic that contributes to this cell's functioning as a trephocyte, for its inability to divide and its tendency toward rapid turnover obligate this cell to altruism. Small lymphocytes can store and transport nucleoproteins and disintegrate or otherwise release this stored material, but they are unable to use these nucleoproteins for mitosis to form additional small lymphocytes.

Small lymphocytes are derived by mitoses of large lymphocytes, a process that occurs primarily in the germinal center of lymphoid tissue. Organs composed primarily of lymphoid tissues and, therefore, essential in lymphocytogenesis, include the lymph nodes, thymus, white pulp of the spleen, Peyer's patches, solitary follicles, and the vermiform appendix.

Plasmacytes and small lymphocytes are only two of a number of the highly differentiated cells that do not divide. However, the absence of mitosis is especially significant with regard to small lymphocytes and plasmacytes because these cells contain nucleic acids, histone, ribonucleic acid (RNA), and other proteins, the very substances essential for division of other cells, yet which do not stimulate division in the small lymphocyte.

Some lymphocytes have the ability to synthesize increased amounts of RNA, gamma-globulin, and other substances in the cytoplasm and, by this means, transform themselves to plasmacytes. Normal or pathological conditions conducive to plasmacyte formation are characterized by stasis of blood, lymph,[90] and/or tissue fluid with increased protein. Plasmacytes also form when there is an increase in available protein, as occurs in limited necrosis in tumors[36] and in other tissues of subacute or chronic inflammatory processes. A number of normal conditions in which there is stasis of protein, as within splenic and lymph nodal sinuses or in the core of intestinal villi, are also places where plasmacytes develop from lymphocytes. The origin of plasmacytes, however, has not been definitely established; although there is abundant evidence indicating that they do develop from lymphocytes, it has not been determined whether small lymphocytes are their only source.

The outstanding characteristic of the mature small lymphocyte is the predominance of the nucleus in proportion to the very small amount of cytoplasm,[59,101] or the high nucleocytoplasmic ratio ("Kern/Plasma" of R. Hertwig). The nucleus of the small lymphocyte of the calf represents about 70 per cent of the cell as compared with the nucleus of liver cells, which represents only 10 per cent.[136] The small lymphocyte, however, has the same amount of DNA as most cells, such as the motor neurons,[162] but does not contain conspicuous cytoplasmic organelles.[59] The difference in function of the large and small lymphocytes may be due to the presence of two types of DNA, as shown by differences in solubility in 9.87 per cent NaCl.[7]

The large nucleocytoplasmic ratio of small lymphocytes is a characteristic that "concentrates" the storage of DNA and the essential amino acids in histone. For example, the rabbit's appendix is composed chiefly of small lymphocytes; it is, therefore, very important as an organ for storage of essential amino acids. Histones from nuclei of small lymphocytes are believed to contain all the essential amino acids except phenylalanine, and only 2 nonessential amino acids, tyrosine and serine.[79]

However, the function of the lymphocyte is not limited to the presence of histone. Tryptophan is held by Mirsky and Pollister[137] to be absent in the histone, but present in the nonhistone protein in nuclei. In addition, the lymphocytes that become plasmacytes contain gamma-globulin and RNA. Gamma-globulin contains more of the nonessential amino acids, and it is of direct functional importance as the primary source of antibodies, and also of indirect importance by furnishing amino acids, or chains of amino acids, that are readily utilized by other cells.

The small lymphocyte is essentially a "packaged unit" that contains a minimum of 17 trephones: phosphoric acid, D-2-desoxyribose, adenine, guanine, cytosine, thymine, adenosine monophosphate (AMP) from DNA, and the 10 amino acids reported to be present in thymus histone.[79] If these components are utilized by other cells as individual entities, the small lymphocytes can furnish all 17. In addition, other cells or processes may use any combination of 2 or more of these trephones until all 17 are used at one time. Considering the possible combinations, to the point where all 17 are used as a single group, there is a possible total (as shown by the formula 2^{n-1}) of 131,071 ways by which small lymphocytes may furnish trephones. Furthermore, transformation of some lymphocytes into plasmacytes adds 7 more trephones (D-ribose, AMP, and 5 additional amino acids in gamma-globulins), thereby resulting in 16,777,205 possible combinations for utilization of trephones derived from the plasmacyte stage of lymphocytes.

The cytolysis of small lymphocytes in certain organs may be a means of furnishing supplemental amino acids for synthesis of proteins in nonlymphoid organs. For example, the amino acids in the histone of small lymphocytes that disintegrate in the spleen or in the hepatic portal vein and hepatic sinusoids could be an important source of amino acids for the synthesis of fibrinogen because most of the amino acids in fibrinogen occur in thymus histone.[79] Furthermore, karyolysis of lymphocytes in the portal organs and in the portal vein could also be a source of many of the amino acids for the synthesis of albumin in the liver.

The small lymphocyte also may be an important source of enzymes. A number of varied enzymes, such as cathepsin, nuclease, amylase, lipase, lysozyme, and adenosinase, occur in lymphocytes of the rabbit.[2] The distribution of alkaline phosphatase in lymphoid tissue has been a controversial subject; certain investigators[32,100] have described lymphocytes and lymphoid tissue as being very rich in this enzyme, but Doyle[47] considered lymphocytes a minor source of alkaline phosphatase, containing only one-twentieth the amount found in reticular cells, and Gomori, in 1954,[48] stated that most of the earlier investigators erroneously believed that lymphocytes have a high concentration of alkaline phosphatase, but that "there is no alkaline phosphatase in lymphocytes." Lymphatic tissue washed free of lymphocytes retained 90 per cent of the alkaline phosphatase activity of the whole tissue, most of which is probably in the reticular cells, although some of it may be in the fibers.[2] Nevertheless, there is evidence indicating that intracellular distribution of alkaline phosphatase coincides with the distribution of DNA in various kinds of cells: for example, oocytes of amphibia,[4] of liver cells,[105] and of bone marrow cells.[150] Danielli and Willmer (1942) found that alkaline phosphatase occurs in chromosomes, while Danielli and Catcheside (1945) and Krugelis (1946) point out that alkaline phosphatase is restricted to the Feulgen-positive bands of the chromosomes.[19] Brachet and Jeener (1956) concluded that phosphatase is found wherever nucleic acid occurs.[155] Arvy and Gabe (1952) noted that phosphatase activity was not related to the content of RNA, and Krugelis (1946) attributed the localization of alkaline phosphatase in the nucleus to the presence of a phosphodiesterase.[155]

Turnover of Lymphocytes

The short life of lymphocytes and their rapid rate of turnover are most significant in considering the quantitative importance of this cell in the synthesis and transportation of nucleoproteins. Some investigators hold that during a 24-hour period more lymphocytes enter the blood stream than one would expect to find in the blood at any one time.[25,49,66,69] Jordan (1939) estimated that in man lymphocytes disappear at the approximate rate of 25 billion per day, and Haden (1940) estimated that the life of a lymphocyte is about 24 hours, thus necessitating a daily production that will replace those lost.[25] McCutcheon[126] thinks that lymphocytes are renewed perhaps as often as 3 times a day, and Drinker and Yoffey[49] cite works indicating that the blood lymphocytes in dogs are replaced an average of more than 3.5 times daily. However, lymphocytes observed through modified Clark-Sandison windows in the ears of rabbits are reported to have lived at least 26 days.[181] Yoffey and Drinker (1939) hold that only about 1 of every 30 of the lymphocytes in circulating blood "finds its way back into the lymphatic capillaries,"[6] while the other 29 lymphocytes arise in the lymph nodes and pass directly into the efferent lymphatics.[182]

Several types of experimental studies have demonstrated that there is a rapid turnover of lymphocytes and that this disappearance of lymphocytes is a normal and regular phenomenon in normal animals. It has been estimated that in the dog about 200 million lymphocytes pass from the thoracic duct into the blood stream every hour.[6,49] Also, it has been shown, by cannulization, that in the dog the average number of lymphocytes in peripheral lymph is constant at about 9040/ml. of lymph.

Bunting and Huston (1921) and Yoffey (1933) calculated the average duration of lymphocytes in the blood from the number passing daily from the thoracic duct into the blood stream and the number normally found in circulating blood.[69] They concluded that lymphocytes spend not more than 4 hours in the blood.[181] Ottesen (1953) suggests that these lymphocytes that leave the blood quickly form a distinct type as opposed to the other type, "which can be indirectly demonstrated in the blood for nearly one year."[69]

The "daily replacement factor (D.R.F.)" for lymphocytes, which is "the ratio of the number of lymphocytes daily entering the blood through the thoracic duct/number of lymphocytes normally present in the blood,"[181] is the logical starting point in organizing an approach to the determination of the actual turnover in lymphocytes. However, it should be kept in mind that, while the D.R.F. represents the major part, it does not represent the total turnover, and values thus obtained would likely be less than the actual value. Those lymphocytes entering the blood stream by routes other than the thoracic duct, those remaining in the connective tissue several days, as held by Clark and Clark (1930),[181] and those entering "the blood stream directly in the lymphoid tissue"[156] cannot be evaluated by the D.R.F. method. Bloom (1938) thinks that most of the lymphocytes enter the blood directly.[156] Unavoidably, all of these lymphocytes are completely ignored in D.R.F. computations.

Yoffey[181] gives the value of the D.R.F. for cats as varying from 0.5 to 3.5, and for rabbits as 5.0. These values suggest a relation of D.R.F. to the total number of lymphocytes in carnivores and herbivores that Brilla[20] believes is statistically significant. Brilla found the average number of lymphocytes in 14 carnivores (wild dogs and foxes) to be 40 to 55 per cent of the white cells, and 65.20 per cent in 17 herbivores (hamsters, mice, and rabbits). However, it should be noted that mice and hamsters are not innate herbivores.

A number of experiments have been devised for the purpose of obtaining longevity values and/or other information on lymphocytes in the blood stream. Since the number of lymphocytes in peripheral lymph and in the blood remains fairly constant while an animal is kept under fairly constant conditions of temperature, nutrition, activity, and general health, the lymphocytic turnover also should be constant.[6,49,66,69,182]

Methods for obtaining information on the lymphocytic turnover are subject to considerable, unavoidable error. Florey (1927) cautions that the number of lymphocytes in efferent lymphatics may be doubled by slight pressure on the lymph node, and Farris (1938) shows that the number of lymphocytes in circulating blood rose appreciably following a brief struggle.[156]

Several investigators have followed the D.R.F. procedure in attempts to determine the number of lymphocytes in the blood before and at varying intervals after ligation of the thoracic duct alone, of one or both cervical trunks or the thoracic duct, or of one or both cervical trunks. Although results were variable, they indicate a reduction of at least 50 per cent in the number of lymphocytes within the first 5 to 12 hours after ligation in dogs, cats, and rabbits.[49] One rabbit showed a loss of 63 per cent in circulating lymphocytes 5 hours after ligation of the thoracic duct and right cervical lymph ducts. A cat lost 56 per cent of its lymphocytes 12 hours after ligation of only the thoracic duct; 19 days after ligation of the thoracic duct its lymphocyte count had returned to the value it showed at the beginning of the experiment.[49]

Fate of Circulating Lymphocytes

Serious consideration has been given to several widely varied explanations for the disappearance of small lymphocytes from the blood stream. Sjövall (1936) believes that small lymphocytes may repeatedly pass from the blood capillaries into the tissue spaces and follow the lymphatics to the lymphoid tissue, whence they set forth again on this continuous cyclic journey.[66,182] Farr[66] lends this explanation a certain amount of support, but states also that it has been shown by Yoffey *et al.*[182] to be definitely untenable since it can account for very few of the lymphocytes that leave the blood. Farr mentions the possibility that some of the 40 million autologous lymphocytes tagged with a fluorescent dye that were injected into rabbits may have been phagocytized, but he points out that his work failed "to produce evidence to substantiate this view."

Another explanation of the fate of lymphocytes is that some of these cells pass out of the blood stream into the red bone marrow where ultimately they become transformed into macrophages, monocytes, granulocytes, and other blood cells.[49,66,91] Cameron[25] supports this idea by pointing out that the number of active mitoses in hematopoietic bone marrow is far too small to replace the daily loss of at least 250 billion red blood cells, and that if the number of lymphocytes that vanish daily were able to change into red cells it would satisfy the discrepancy in in red cell turnover.

Tissue-culture methods, however, do not indicate that small lymphocytes transform into monocytes and that monocytes develop into fibroblasts.[73,110] Likewise, Ebert *et al.* (1940), after constantly viewing cells in a rabbit's ear through a modification of the Clark-Sandison chamber for 24 hours, reported that they saw nothing to indicate that small lymphocytes are converted into other cells.[25] Similar results have been reported by various investigators using the transparent chamber technique on rabbits' ears in which small lymphocytes remained alive as long as 26 days without apparent transformation to other cells.[181] A few lymphocytes may develop into plasmacytes, but only under conditions of nearly complete stasis, which increases the plasma protein or which involves conditions such as edema.[97] Normally, the rabbit's ear would be expected to fail to meet these conditions. Unless subchronic or chronic inflammation is present, it is possible that other lymphocytic transformations may require conditions that cannot readily be met by *in vitro* methods for, as Cameron[25] points out, lymphocytes have been reported to develop into myelocytes in bone marrow *in vivo*, but not *in vitro*.

The removal of blood lymphocytes in the liver and spleen has not been refuted. This idea is supported by syngenesic transfusion of P^{32}-labeled lymphocytes into rats and rabbits.[69] Weisberger *et al.* (1951) hold that lymphocytes are "filtered out of the circulation in the lungs."[69] The tenet that small lymphocytes disintegrate in the blood appears to have the support of certain investigators, most of whom relate the disintegration to release of gamma-globulin,[6] desoxyribonucleic acid phosphorus,[69] and other products. Drinker and Yoffey,[49] however, cite works that indicate that the majority of the lost lymphocytes do not disintegrate in the blood.

Emigration into the lumen of the stomach and intestines is one of the oldest explanations of "the vanishing lymphocyte." Although small lymphocytes occur frequently within the columnar epithelial cells of villi, they very rarely pass into the lumen. These small lymphocytes in the intestinal columnar cells usually occur between the basement membrane and the nucleus; for example, 96.4 per cent of the 6595 lymphocytes counted in the epithelial cells covering villi were between the nucleus and basement membrane.[93] Lymphocytes sometimes pass into the lumen through the lymphoepithelium covering the follicles, Peyer's patches, and other intestinal lymphoid tissue, although the percentage of the total surface area that consists of lymphoepithelium is very small. The number of lymphocytes free in the lumen of the small intestine also is related to the extent of autolysis of intestinal mucosa and to the speed and method of fixation.[93] Many more lymphocytes were present in the lumen of the intestines in which autolysis was present than in those in which the lumen was immediately distended with fixative.

Labeled lymphocytes have been transfused into mammals in attempts to determine the distribution and fate of blood-borne lymphocytes. Fichtelius[69] injected rats and rabbits with radioactive phosphorus (P^{32}); after the P^{32} had been incorporated with the DNA of lymphocytes to form $DNAP^{32}$, the animals were sacrificed and the lymphocytes of the thymus (thymocytes) were intravenously transfused into the experimental animals, which were killed after 24 hours. Radioactively labeled lymphocytes were observed in the mucous membrane of the small intestine, as well as in the spleen, thymus, lymph nodes, liver, lungs, and bone marrow.[69]

Farr[66] injected rabbits intravenously with a fluorescent dye (3,6-diamino-10-methylacridinium chloride in Tyrode solution, 1:1 million) and, on the basis of his experiments, concluded that most of the lymphocytes that leave the vlood vessels go directly to either the lymphoid tissue or to the bone marrow. Two hours after the injection, labeled lymphocytes were found in the lymphoid tissue, including the "mesenteric lymph nodes," Peyer's patches, vermiform appendix, thymus, white pulp of the spleen, and bone marrow. Twelve hours after the injection, most of the labeled lymphocytes had disappeared from these structures, except in the lymphoid tissue. Farr also occasionally found a labeled lymphocyte in the submucosa or lamina propria of the intestine and in connective tissue in various parts of the body. Heterologous, labeled guinea pig lymphocytes could not be found in any organ at either 2 or 12 hours after injection into rabbits.[66]

PLASMACYTES

The plasmacyte (plasma cell, plasmocyte, Russell body cell) seldom occurs in normal peripheral blood, except in cases of measles (especially German measles), scarlatina, chicken pox, and plasma-cell leukemia.[178] The terms "infiltrate" and "aggregate," although in common usage, are not in order when applied to plasmacytes; this cell, unlike the lymphocyte and certain monocytes, is apparently incapable of locomotion or of being deposited within the tissues by the blood. Various stages from lymphocytes to fully formed plasmacytes occur in normal and pathological tissues. Older writers loosely refer to this condition as "round cell" infiltration, a term that is considered objectionable in the more recent literature.[152]

Many years ago several investigators proposed that plasmacytes had important functions in supplying nutritive material. Enderlen and Jurti (1901) and Porcile (1904) held that "the cells were carriers of nutritive material," and Dantschakoff (1905) maintained that plasmacytes in the submaxillary gland absorbed substances from the blood and lymph that they transmitted to the alveolar cells.[134] More recently, various types of experimental procedures have indicated that the plasmacyte is a source of antibody. However, it has not been unequivocably established that the plasmacyte is the only source of antibodies or that the formation of this antibody is its only function. Numerous relations indicate that the plasmacyte has a more basic function in the synthesis and storage of globulins and RNA and that the formation of antibodies represents only one manifestation of its basic function of protein synthesis.

The outstanding cytological change that occurs during the transformation of lymphocytes to plasmacytes is an increase in cytoplasm that has a high content of RNA. Basophilic cytoplasm is one of the outstanding characteristics of plasmacytes[5,80,88] and was the characteristic that first attracted the attention of Ramon and Cajal a year before the cells were described by Unna.[135] Unna thought that the basophilia of plasma cells was their outstanding characteristic, rather than the arrangement of the nuclear chromatin, as proposed by Marschalko.[134] Zylbersza (1941) stated that the intense basophilia of plasmacytes was due to ribonucleoproteins.[8] However, Ferrata pointed out that cytoplasmic basophilia is not peculiar to plasmacytes.[134] Basophilic cytoplasm also has been reported in other cells.[87]

Ribonucleic acid accounts for a considerable amount of the cytoplasmic basophilia of plasmacytes.[17] Harris and Harris[74] found that RNA appeared in developing plasmacytes in rabbits on the second day after injecting the antigen. Use of the RNAse histochemical test and a solution of methylene blue in 4.9, a method considered specific for RNA,[72] shows that plasmacytes contain more RNA than occurs in the cytoplasm of most cells, even including hepatic, pancreatic alveolar, and Purkinje neurons; these cells are thought to have higher concentrations of this nucleic acid than are found in the cytoplasm of most mature, nondividing cells in tissues of the higher vertebrates.

The earlier descriptions of the granular nature of the cytoplasm of plasmacytes have been confirmed by use of the electron microscope. In 1931 Michels[134] reviewed the ideas on presence of granular cytoplasm and stated that Kingsley (1924) mentions a "homogenous granuloplasm" as the first sign in the formation of plasma cells from lymphocytes. Jadasson held that the cytoplasm was crumbled rather than granular. DeAsua (1922) states that the crumbled form is the result of an accumulation of secretion.[134] Ehrich[57] stated that studies with the electron microscope show that the cytoplasm of plasmacytes consists of a bundle of filaments that is "a framework of ribonucleoprotein that is the factor which produces protein for export." Cytoplasmic basophilia, as interpreted from studies using electron micrographs, is considered to be due to long parallel or concentric—sometimes branching—lamellae, which may contain RNA.[38] Electron microscope sections by Braunsteiner *et al.*

(1953) and Policard *et al.* (1954) show that the cytoplasm of plasmocytes (a term used synonymously with plasmacyte) is composed largely of archoplasmic lamellae,[8] which develop dilated sacs when the plasmacyte develops into a Mott cell.[8] Palade[145] found granules that were usually spherical, had a relatively high electron density, measured 80 to 300 Å in diameter, and were abundant in the cytoplasm of plasma cells, as well as in other cells having an intensely basophilic cytoplasm, such as liver cells and acinar cells of the salivary, mammary, and pancreatic glands. The similarity of the granules in the alveolar cells of salivary and mammary glands and pancreas to those occurring in the cytoplasm of plasmacytes is especially significant in view of the fact that plasmacytes occur in the stroma of these glands and may form the granules or may provide the RNA or its components.

Plasmacytes do not use cytoplasmic proteins for mitosis. The absence of mitosis in plasmacytes is recognized by most hematologists,[49,122,134] while others[134] report amitotic division with production of multiple nuclei. Michels[134] reviews the earlier observations on the absence of mitosis in plasmacytes and states that Cajal noted that mitosis is rare. Verrati and Krompecher attributed the formation of multinucleated plasmacytes to direct nuclear division, and several authors considered amitosis as the cause of binucleate and trinucleate plasmacytes.[134] However, Jordan[89] holds that "young plasmacytes in lymph nodes divide by mitosis, older plasmacytes divide by amitosis," giving origin to cells having two or more nuclei. Since the origin and identity of "young" plasmacytes is uncertain, it is possible that the question of dividing mature plasmacytes may be resolved into a case of mistaken identity as to the stage of development or the kind of cell, since there are wide morphologic variations in plasmacytes and close resemblances to other types of immature ("blast") blood cells.[49,68,122,181]

The relation of RNA to protein synthesis has been established by many investigators using diversified techniques. Both Brachet (1942) and Caspersson (1941) concluded, from studies in which cytochemical methods were used, that RNA had a function in protein synthesis.[18] The later literature on the cytochemical and quantitive evidence for RNA in protein synthesis is reviewed by Brachet.[18] Microspectrographic methods have shown that protein synthesis occurs in cells with high concentrations of RNA in the cytoplasm and nucleus.[30] Caspersson[30] believed that the appearance of the ribonucleotides in the nucleolus and cytoplasm occurs during protein synthesis, but Brachet (1950) thought that protein synthesis is closely related to cytoplasmic RNA.[44] In 1946 Schneider demonstrated that the bulk of the RNA in homogenates of rat liver was located in the unfractionated residue, which includes the microsomes.[40]

However, the exact method by which RNA participates in protein synthesis has not been determined. Brinkley was of the opinion that RNA has a part in peptide synthesis.[40] RNA also has been believed to be a possible source of energy-rich bonds by Spiegelman (1946).[18] Borsook[12] reviews theories on functions of the nucleic acids in protein synthesis and points out that RNA has a more direct part than DNA. He considers the possibility that the carboxyl group may be linked to a phosphate and that the amino group remains free until formation of the peptide bond in protein synthesis by the template hypothesis.

At the present time results of varied experimental methods indicate that plasmacytes (plasma cells) are the primary source of antibody. Plasmacytes increase in the medullary cords of lymph nodes following injection of antigens.[59] Antibodies have been extracted from some of the tissues in which plasmacytes increased after injections of bacteria,[74] and also from explants of spleen, which also contained increased numbers of plasmacytes after bacterial injections into rabbits (Fagraeus).[74] McMaster[127] considers the controversial status of the reticuloendothelial cells and states that, until better identification of plasma cells is made and the relation of lymphocytes to plasmacytes is determined, the problem will remain confused. However, Ehrich[58] no longer considers the reticuloendothelial theory of antibody formation correct. Many of the more recent reviews on antibodies represent the plasmacyte as the most likely source of antibodies.[24,173,181] Plasmacytes have been considered the source of globulins, precipitins, and agglutinins.[57] Plasmacytes also were considered an important source of antibody and serum globulin by Fagraeus (1948).[24] In addition, the number of plasmacytes has been reported to parallel the serum antibody.[104] However, Burnet and Fenner[24] state that future work may modify wholehearted acceptance of plasmacytes as the source of antibodies.

Lymphocytes, now considered one source of the cells identified as plasmacytes, formerly were considered the important source of antibodies. Lymphocytes were called "a storehouse of antibody protein" by Dougherty *et al.* (1944).[26] This cell, which also has been considered a source of substances for the synthesis of nucleic acids and proteins[58] and for phosphorylation and energy,[58] likewise has been believed to release gamma-globulin upon disintegration in the germinal follicles of lymphoid tissue.[168] Relations of lymphocytes to foreign proteins were reviewed by Rich *et al.*[153] who also reported original work in which injection of foreign protein caused lymphoid proliferation.[26] The problem is further complicated by reports that the "immature" plasmacyte is active in antibody formation.[98] Since some, if not all, cells identified as plasmacytes develop from lymphocytes, a sharp distinction between lymphocytes and plasmacytes cannot be obtained because there is one step at which classification of the cell as a lymphocyte or plasmacyte is questionable. Young plasma cells, which are sometimes considered lymphocytes,[15] are recognized by Weiss[172] as the main producer of antibodies. In addition, the possibility has been mentioned that the lymphocyte may be the cell to which globulins are transferred to receive the antibody stamp.[24]

A review of the literature shows that most investigators concur in the idea that antibodies are gamma-globulins, and that plasmacytes produce globulins because this cell increases in conditions of hyperglobulinemia[57,133] and decreases or is absent in conditions of agammaglobulinemia.[23,71,183] Utilization of injected ribonucleotides has been considered a cause of hyperglobulinemia in rabbits[154] and as being related to antibody formation by Fagraeus (1948).[181] A peak of RNA in popliteal lymph nodes of rabbits that received injections of typhoid antigens into the foot pad coincided with maximal formation of antibodies.[59] Antibodies occur primarily in the gamma-globulin fraction[76,144] and are classed as globulins.[121] Injections of various antibodies and serum into rabbits show that horse serum globulins cannot be differentiated from antibodies in horse serum.[78]

Plasmacytosis is commonly associated with hyperglobulinemia.[127] Plasma cell myelomas are accompanied by high blood globulin levels and produce "all sorts of abnormal proteins."[138] In addition, protein deficiency,[26] prolonged administration of cortisone,[46,70] irradiation,[163] and nitrogen mustards[160] decrease plasmacytes and also reduce the amount and rate of antibody formation.

More recent studies indicate that the difference in the structure of antibodies and globulins may be very slight. Preliminary work shows that the sequence of amino acids is the same in antibodies and globulin.[78] Another idea of the structure of antibodies is that normal globulins and antibodies differ by the mode of folding of the peptide chain.[77] Antibodies also have been considered to differ from normal serum globulin only in the manner by which the two ends of the chain of the globulin polypeptide are coiled,[75] instead of the arrangement of amino acids, which was formerly considered the method by which antibodies and globulins differ.[26] In addition, these similarities give further evidence for the functional significance of plasmacytes, in that this cell may be, not only a source of antibodies, but also an important source of gamma-globulins. Putnam and Miyake[149] also have thought that cryoglobins, which are abnormal plasma globulins, differ from each other and from normal gamma-globulin only in the amino acids on the N-terminal position. Analyses of 20 myeloma globulins were divided into 5 types, based upon differences in the N-terminal amino acid.[148]

Synthesis of proteins other than antibodies has been attributed to plasmacytes because of the concentration of RNA, which is more abundant where synthesis of protein is intense, and because the electron microscopically visible structures that resemble those occurring in protein are present in plasmacytes, but are absent in lymphocytes.[57] Furthermore, Liew *et al.*[15] found that immunization of rabbits with pneumococci produced an increase of globulin only one half to two thirds of which was antibody. Burnet and Fenner[24] have suggested the possibility that the cell responsible for antibody production may be the same cell that produces serum globulin, and Terroine[165] states that, during immunity, RNA in plasmacytes may "govern a proteinogenesis of which the liberated products would contribute to the formation of blood plasma."

NORMAL DISTRIBUTION OF PLASMACYTES

Several normal tissues contain considerable numbers of plasmacytes. Lymphocytes develop into plasmacytes in tissues where vascularity causes stasis or increased concentration of plasma proteins. These conditions obtain in sinuses in lymph nodes, in sinusoids in the spleen, and in the core of intestinal villi of some species on a high protein diet. Plasmacytes are present singly or in small numbers in the connective tissues of most organs, but plasmacytes are very abundant and comprise most of the cells in several tissues. Numerous investigators have shown that plasmacytes are significantly abundant in normal fishes, amphibia, reptiles, birds,[134] and mammals.

Differences in the number of plasmacytes in connective tissue of various normal organs show a correlation between the amount of RNA in cells of the organ and the increased presence of plasmacytes in adjacent connective tissue. For example, the submaxillary and parotid glands, where alveolar cells also contain great amounts of RNA, have plasmacytes scattered throughout the connective tissue. Furthermore, many of these plasmacytes are disintegrating, as indicated by vacuolation of the cytoplasm, a decrease in RNA, and fragmentation of the cytoplasm. It is interesting to see that, as early as 1905, Dantschakoff believed that plasmacytes in the submaxillary gland retained substances from the blood for later use by alveolar cells.[134]

The number of plasmacytes in some normal organs varies during changes induced by hormones. Scattered plasmacytes are usually present in the interalveolar and interlobular connective tissue of the mammary gland at all times, but these cells do not "infiltrate" or "aggregate" until growth, hyperplasia, or involution occurs in the mammary gland. The increase in lymphocytes and plasmacytes at this time insures a more adequate supply of nucleic acids, histones, and other proteins for the increased proliferative or functional activity of stromal and secretory cells than could be obtained from the hyperemic blood vessels alone.

The infiltration of lymphocytes and plasmacytes in the mammary gland at the termination of lactation has been considered a resorptive mechanism, usually for decomposed material or breast secretion.[14,108,164] Chronic inflammation resulting from milk stasis is often held to be the cause of the infiltration, but several authors recognize the fact that the reaction is not due to infection.[147] Lymphocytes are also considered to have a function in the removal of secretions and disintegrating cells.[14] Taylor[164] considered "round cell infiltration" as more likely related to resorption and secretion than to an inflammatory reaction.

Infiltrations of lymphocytes and plasmacytes in the mammary gland precede colostrum secretion at the beginning of lactation. The coincidence of infiltration of these cells with colostrum formation[115] is partly responsible for the belief that the plasma cells are colostrum corpuscles. Furthermore, the disintegration of plasmacytes within the lumen of alveoli might explain the extremely high content of gamma-globulins and, in turn, of antibodies in milk[147] during the period of colostrum formation and the subsequent decrease in these substances during the period of lactation, when there is relatively little infiltration into the stroma by lymphocytes and plasmacytes.

Lymph Nodes and Patches

Plasmacytes develop in normal lymph nodes that have afferent and efferent lymphatics connected by sinuses. The plasmacytes occur primarily in the medullary cords.[89,90,124] Jordan and Morton[90] call attention to the fact that "the great abundance of plasma cells in the medullary cords of certain nodes" in normal animals "has been largely overlooked or ignored," but Jadassohn (1891-1893) has demonstrated plasmacytes in lymph follicles in man and lower animals.[134] Various investigators also have shown that plasmacytes are common in the medullary cords in the mesenteric nodes,[124] in the submaxillary lymph nodes of rats,[89] and in the retrothymic nodes of hamsters.[97] Jordan[89] calls attention to the conditions in which the amount of lymph supply and lymph stasis, or edema fluid, may be a necessary factor for the transformation of lymphocytes to plasmacytes. Furthermore, he reported that plasmacytes occur in the omentum and intestinal mucosa, and may appear scattered or as aggregates in the marrow.

The retrothymic lymph nodes, which receive lymphatics from the thymus, contain more plasmacytes and have a larger area of medullary cords in proportion to size than most other lymph nodes of hamsters that have afferent and efferent lymphatics. Plasmacytes, however, are rare within the thymus.[97]

Plasmacytes are very significant components in the core of villi of some animals, and also in the mesenteric lymph nodes of herbivorous, as well as carnivorous animals, but they occur rarely in intestinal lymphoid tissue. Since plasmacytes are abundant in the core of villi of carnivorous and omnivorous animals, it is difficult to ascertain whether the plasmacytes arise from lymphocytes in mesenteric lymph nodes or whether they are carried as cells in the lymphatics from the lamina propria, especially from the core of villi, to the mesenteric nodes. In rabbits, however, most plasmacytes in the pancreas aselli (a group of three to seven large lymph nodes in the mesentery situated at the base of the multiple branches of the superior mesenteric arterial trunk) might be considered to arise from lymphocytes, because plasmacytes are not abundant in the core of villi and are very rare in the intestinal lymphoid tissues in this animal.

Several investigators[41,89,90] have considered that lymph stasis may furnish an important stimulus for transformation of lymphocytes into plasmacytes. The plasmacytogenic benefits derived from stasis appear, however, to be due to an increase in protein rather than to the "formation of noxious metabolites"[89,90] or to "some form of toxin," as some investigators[89,90] appear to believe.

Hemolymph Nodes

Plasmacytogenesis occurs in certain lymphoid structures that are interposed in the blood vascular system and that are designated hemolymph, or hemal nodes. However, the structural relations of hemal nodes to the blood and lymph vessels apparently have not been determined in sufficient detail to make definite differentiation practicable in all nodes examined. Plasmacytes occur in the medullary cords of hemolymph nodes,[90,119] where they are surrounded by blood and/or lymph. Jordan and Morton[90] noted that sinuses of nodes that appeared to be hemal nodes contained blood and cells "predominantly of the plasma cell type." MacMillan[119] reviewed the literature on hemal nodes and concluded that plasma cells normally occur in hemal nodes of the rat, guinea pig, and sheep. Jordan[89] does not mention hemolymph nodes as a site for plasmacytogenesis, but he states that "it seems probable that in the spleen, thymus, bone marrow, omentum and intestinal mucosa plasmacytes arise only from lymphocytes."

Fluid in sinuses of hemal nodes has not been definitely identified. Some investigators hold that the sinuses of hemal nodes contain a mixture of blood and lymph or alternately contain blood or lymph, while others maintain that only blood is present. Lewis[109] states that blood and lymph are mixed in hemolymph organs in contrast to the spleen, in which blood is the only fluid. Weiss[173] states that the characteristic of hemal nodes is the presence of blood in the lymph sinuses, and that these nodes lack efferent and afferent lymphatics, but apparently have the sinuses flooded with blood. From the standpoint of stasis of proteins as the factor in plasmacytogenesis, the question whether the fluid in hemal nodes is blood or blood and lymph is immaterial, provided excess protein is present.

Several functions have been ascribed to the hemolymph node, but its significance in protein metabolism and as a site for the development of plasmacytes has not been recognized. Hemolymph or hemal nodes have been regarded as hematopoietic organs. Erencin[64] concluded that myelopoiesis is normal in hemal lymph nodes of sheep and goats, but MacMillan[119] holds that hematopoiesis does not occur in hemal nodes in sheep, guinea pigs, or rats. Lewis[109] points out that "it seems highly improbable that newly formed cells should be freed in the sinuses to be promptly destroyed." Woltmann[179] held that the hemolymph nodes play some part in the production of eosinophils, and Warthin (1901) believed that hemal nodes had a hematopoietic function in several pathological conditions.[119]

Hemal nodes have been classed as lymphoid tissue[179] and have been considered organs for the destruction of blood cells in birds.[109] Paton *et al.* considered that the hemal and hemolymphatic nodes were the normal site for hemolysis and that hemolysis in the spleen occurred only under abnormal conditions.[109] Lewis[109] holds that destruction of erythrocytes, eosinophils, and polymorphonuclear leukocytes, as well as a limited destruction of lymphocytes, occurs in all hemolymph nodes.

Destruction of various blood cells in the hemolymph nodes is important in evaluating conditions under which lymphocytes become plasmacytes, because cytolysis of various blood cells increases the hydrolytic products of nucleic acids and other proteins, such as the amino acids in globin from erythrocytes. Necrosis of cells has been shown in some cases to provide growth stimulants for other similar cells.[36] This suggests that the lysis of blood cells in hemolymph nodes and in the spleen increases the nucleic acid and amino acids above those normally found in blood and, in this manner, favors plasmacytogenesis. The synthesis of increased RNA and globulins in plasmacytes, therefore, resynthesizes and stores protein in these organs.

PLASMACYTES IN PORTAL ORGANS

Numerous and varied functions in protein metabolism have been attributed to the liver, but the significance of protein storage by lymphocytes and plasmacytes in the spleen and other portal organs to the several functions of the liver in synthesis of plasma proteins is not usually considered. Lymphocytes and plasmacytes also occur in the mucosa of the stomach, small and large intestine, and in "milk spots" and lymph nodes in the mesentery, as well as in the spleen. The liver, therefore, is the first organ to have access to the nucleic acids, histones, and globulins derived from lysis of lymphocytes and plasmacytes in the portal organs and/or vessels. The spleen, however, is the only portal organ that has a well-developed mechanism for permitting the normal entry of whole plasmacytes directly into the hepatic portal vein; lymphocytes in the portal organs can readily enter the hepatic portal vein or pass through central lacteals, mesenteric lymphatic system, and thoracic duct to enter the systemic venous blood in the precaval system. Thus, entire lymphocytes and the substances released by lysis of lymphocytes and plasmacytes may pass over the hepatic portal tributaries to the liver or over the lymphatics to mix with the systemic blood.

Spleen

The spleen is often considered the only lymphoid tissue interposed directly in the blood stream,[109,125] since the relationship of hemal nodes to the blood and lymphatic systems has not been definitely established. Venous sinuses in the spleen are places where lymphocytes and monocytes enter the blood[125] and are also regions in which plasmacytes are abundant.[94,96] The venous sinuses of the spleen afford areas where pooling or limited stasis of blood occurs, where protein may be stored as gamma-globulin in the cytoplasm of plasmacytes, and where the plasmacytes subsequently may disintegrate to furnish proteins in the hepatic portal vein.

The spleen offers conditions highly favorable for normal, or nonpathological, transformation of small lymphocytes into plasmacytes in hamsters.[94,95] Thus, lymphocytes that are retained in the Malpighian bodies of the spleen and those in splenic sinusoids can synthesize and store nucleoprotein and other essential substances within the blood vascular system. The red pulp of the spleen is a region in which developing plasmacytes are in contact with protein in the blood and also a region where protein is increased by cytolysis of leukocytes and erythrocytes, which processes locally increase the plasma protein level above that of blood in most organs.

Pappenheim's term "splenocyte" is limited to a large migrant, mononuclear phagocyte,[45] but the term "splenic cells" or "splenic corpuscles" includes both the plasmacytes and monocytes. Recently, plasmacytes have been recognized in the red pulp of the spleen, but most earlier investigators appear to class plasmacytes found among the mononuclear cells as monocytes, splenic corpuscles, or mononuclear or splenic cells. Weiss[173] states that plasmacytes are especially abundant in the red pulp. Ehrich[57] also recognizes the presence of plasmacytes in the red pulp of the spleen. Jordan[88] supports this point by stating that plasmacytes may be present in considerable numbers in the red pulp of the spleen. Maximow and Bloom[125] recognize the presence of plasmacytes in the red pulp of the spleen in the mouse, guinea pig, hedgehog, rabbit, man, and human embryo.

Plasmacytes not only form in the sinuses of the spleen, but they also disintegrate within them, as well as in the splenic and hepatic portal veins. The results of preliminary[97] and other work[94] indicate that lysis in the blood stream of plasmacytes arising in the splenic sinuses appears to be a function of enzymes produced by the liver and portal organs other than the spleen. Normal systemic blood of man[178] contains few to no plasmacytes. In hamsters, plasmacytes are abundant in the venous sinuses of the spleen, which are interposed directly between the systemic arterioles and venules of the hepatic portal system, but they are very rare in blood from the jugular vein.[94]

The disintegration of plasmacytes in the blood appears to be a normal process, since comparisons of four control and sixteen tumor-bearing hamsters show no significant differences in the number of plasmacytes entering the systemic blood.[94] This destruction of plasmacytes in the hamster agrees with the statement of Maximow[122] that "their ultimate fate seems always to be degeneration." The disintegration of plasmacytes within the spleen and the splenic and hepatic portal veins in hamsters may be an important factor in the protein metabolism of hepatic cells, especially as indicated by their RNA-produced cytoplasmic basophilia.[94,96] The liver, which is usually considered a source of several plasma proteins,[76,174] might be expected to concentrate these substances from the hepatic portal vein.

Intestinal Lymphoid Tissue

Formation of lymphocytes and plasmacytes in the mucosa of the small and large intestines represents a synthesis and storage of protein that is interposed between the lumen of the intestines and the hepatic portal vein. Small lymphocytes occur as aggregates in the intestinal lymphoid tissues, which include Peyer's patches, cecal lymphoid tissues, and the appendix, and as single cells in the columnar epithelium of villi.

Intestinal lymphoid tissue occurs in the mucosa of the small and large intestines and includes solitary follicles, Peyer's patches, and various arrangements of lymphoid tissue in mucosa of the cecum or in the vermiform appendix. The intestinal lymphoid tissue does not have typical afferent lymphatics. The Peyer's patches in the small intestine of mammals consist of aggregates of three or four solitary follicles. Scattered, typical solitary lymph nodules occur in the lamina propria and/or mucosa of the mouth, esophagus, stomach, and small and large intestine.[11]

Most of the intestinal lymphoid tissue consists of numerous small areas dispersed throughout the length of the intestines, but the appendix of the rabbit is very large, is composed primarily of lymphocytes, contains a very small amount of connective tissue, and occurs at the lip of the cecum, a location that facilitates studies on effects of extirpation. The appendix of adult rabbits of the medium-sized breeds, such as New Zealand Whites, averages from 4 to 4.5 inches in length and has a large open lumen. The wall of the appendix of rabbits is composed primarily of lymphocytes and contains very little connective tissue, in contrast to the great amount of connective tissue and the small isolated lymph nodes in the normal human appendix. The rabbit appendix is therefore about 1 inch longer than the actual average length of the human appendix[82] and 60 times the size of that of man when consideration is given to differences in total body size.

This large appendix of the rabbits, with its great amount of lymphoid tissue, in contrast to the small lymphoid patches in the cecum of rats, guinea pigs, mice, and other rodents, apparently provides a specialized site for the formation, absorption, and storage of nucleic and amino acids. Rabbits require much less protein in their diet (which consists of a relatively larger proprotion of cellulose) than do most mammals.[157] The cecum of New Zealand White rabbits, which is about 2 feet in length and contains a spiral-like valve, is an organ in which cellulose is hydrolyzed and in which bacteria profliferate and die. The amino acids and other components of proteins could be absorbed through the lymphoepithelium forming the bottom of the flask-shaped mucous glands of the appendix and possibly stored as histones in the nucleus of small lymphocytes in the lymphoid nodules. Some small lymphocytes that are produced in the appendix pass into lymphatics, and some go to the pancreas Aselli, where they may be stored for longer periods. Many small lymphocytes that are produced in the rabbit appendix pass into the hepatic portal vein and through the liver, thereby becoming available to all tissues.

Bacterial proliferation in the cecum and other parts of the large intestine of rabbits also increases the protein content of the diet by coprophagy, which is a normal practice in rabbits. Decomposition of bacteria in the cecum provides protein components that pass in the thin, alkaline fluid into the appendix, while the remaining solids are "screwed" back into the colon by action of the spiral-like valve. In many rodents the cecum does not have an important function because it is relatively shorter or smaller and because peristalsis forces material into the colon in a short time.[10] In addition, the protein synthesis of B vitamins by the intestinal flora, which has been established by various methods,[142] also would be a function of the cecum.

Large lymphocytes in the germinal follicles of Peyer's patches and solitary follicles also synthesize and store histones from amino acids during the formation of nucleoproteins. Most descriptions of the absorption of amino acids state that the amino acids that enter the body are carried by the blood to all tissues, where they become incorporated.[79] However, those amino acids that pass through the lymphoepithelium covering the intestinal lymphoid patches are directly and immediately available for use in the synthesis of histones by the large lymphocytes in the germinal centers of the intestinal lymphoid tissues. The formation of nucleoproteins in the nuclei of lymphocytes and in the cytoplasm of plasmacytes incorporates significant quantities of the dietary amino acids directly from the lumen of the intestine without their having passed into the blood. Thus, the numerous lymphoid patches and aggregated cells in the mucosa of the small and large intestines have direct access to dietary amino acids and other substances necessary for the synthesis of nucleoproteins. The significance of this provision becomes apparent when one considers the amount of tissue contained in the total number of solitary follicles and Peyer's and other intestinal lymphoid patches.

Protein synthesis in large lymphocytes, which occurs primarily in the germinal centers of the lymphoid structures, represents an incorporation of the amino acids that pass from the intestinal lumen (through the lymphoepithelium into the lymphoid tissue) into the histone, which is combined with the DNA in nuclei. Most of the cells produced by mitosis of large lymphocytes become small lymphocytes, which do not divide and, therefore, are not utilizing the contained amino acids for proliferation of their kind, and which may transport this protein when they leave the intestinal lymphoid tissues through either the veins or the lymphatics. Small lymphocytes that enter lymphatics are stored for a longer period in the pancreas Aselli or other mesenteric lymph nodes. Those entering the portal venous system pass into the systemic circulation a little sooner than those taking the lymphatic route through the thoracic duct. Small lymphocytes, the only blood cells normally present in all circulatory and tissue fluids, disintegrate constantly in large numbers in the blood, lymph, and tissue fluid: a process whereby the lymphocyte releases amino acids as histones, as well as derivatives of nucleic acids.

Plasmacytes in Villi

The conclusions of several investigators[9,60,85,106] to the effect that plasmacytes are related to the formation of globulin and to the general problem of protein metabolism indicate that changes in the number of these cells in the intestinal villi may be significant in protein metabolism. Plasmacytes in the core of villi increase during the period of digestion.[124] Intestinal villi of rabbits and man have a special arrangement in the form of networks of precapillary arteriovenous anastomoses throughout each villus,[84] which is aided by the inherent motility of the villus and which virtually prevents stasis in the normal villus. Plasmacytogenesis in the core of villi would appear to be a response to absorbed dietary protein rather than to exudation.

The number of plasmacytes in villi of the small intestine varies in different species. For example, plasmacytes are abundant in the core of intestinal villi of hamsters, but are much less numerous in the villi of mice. Also, the actual numbers of plasmacytes and their distribution within a single villus vary with changes in the form and location of the villus throughout the length of the small intestine and with regard to proximity to a Peyer's patch, and also in different, although comparable, individuals in an inbred colony of hamsters. Further work[37] with hamsters from this colony showed that plasmacytes were much more numerous and more evenly distributed in villi of the posterior half of the duodenum than in the jejunum or ileum, except possibly in the last inch of the ileum, where they are usually more numerous.

Starvation variously affects plasmacytes in the core of villi. Six hamsters having water ad libitum, but no food for 3 to 5 days, had notably reduced plasmacytes in the core of the villi of the duodenum and ileum.[37] Ten hamsters, starved until near death of 1 in each of 2 groups, showed a general depletion of plasmacytes in the core of the villi; however, since comparison of plasmacytes was not a part of the problem, it was not considered in detail.[128] The number of plasmacytes was decreased in the ilea of all 15 hamsters maintained for 10 to 36 days on an otherwise adequate synthetic protein-free diet, to which 2 per cent brewer's yeast was added. However, the plasmacytes in the duodenum were reduced in only 4 of this group.[37]

The number of plasmacytes in villi varies in systemic conditions that alter nitrogen retention. On the one hand, human gamma-globulin administered subpannicularly in 6 to 20 injections of 0.5 to 1.0 ml. over a period of 6 to 47 days greatly increased plasmacytes in the villi of the duodenum and in many villi in the ileum in 5 hamsters.[37] On the other hand, cortisone acetate (10.0 mg./kg.) injected subpannicularly 3 to 18 times into 13 hamsters during a period of 5 to 41 days reduced plasmacytes and caused dilation of the lacteals in the villi of all 13. X irradiation of the entire body, which rapidly depleted the Peyer's patches, more slowly destroyed the plasmacytes in the core of villi. For example, the Peyer's patches were depleted in 25 of 29 irradiated hamsters, but the plasmacytes in the core of the villi remained normal in 13 of the 25 and were depleted in 12 animals. This correlation corresponds to the ratio of depletion in peripheral lymph nodes. In irradiated hamsters the cortex of lymph nodes decreased rapidly and to a great extent, while the medullary regions of the nodes remained normal in size for several days after depletion of the cortex. However, in the cortisone group, the decrease in the size of the cortex and medullary cords occurred at approximately the same rate and to the same degree.[37]

Lymphocytes in the Columnar Epithelium

Small lymphocytes in the cytoplasm of columnar epithelial cells of villi in the small intestine may have a function to compensate for the modifications in the nucleoprotein metabolism of columnar cells of villi. The epithelial cells that cover villi are produced by mitosis in the crypts of Lieberkühn. Epithelial cells of villi are active in assimilation of proteins from the lumen of intestine, but have become specialized and do not divide. This modification facilitates passage of amino acids and other proteins from the lumen of the intestine through the epithelium.

Small lymphocytes occur in the cytoplasm of many columnar epithelial cells that cover villi in the small intestine. Lymphocytes frequently emerge into the lumen through lymphoepithelium of Peyer's patches and solitary follicles, which comprise a very small percentage of the surface area of the intestines. In hamsters, however, only 3.6 per cent of the intracellular lymphocytes in columnar cells of the ileum lay between the nucleus and the striated border, while 96.4 per cent of the 6595 cells counted occurred in the basal third of the epithelial cells. Only rarely did the position of a lymphocyte indicate emergence from a columnar cell of a villus.

Small lymphocytes in the cytoplasm of columnar epithelium have not been observed to be associated with an increase of DNA, RNA, or of protein in the columnar cells that contain small lymphocytes. However, no conclusions can be drawn from this observation because, without studying serial sections, it is not possible to determine whether a columnar cell contains small lymphocytes. Furthermore, it is impossible to determine if a small lymphocyte has recently withdrawn from the cytoplasm of a columnar cell into the core of villi. In addition, if the small lymphocyte does release particles of DNA, it would not be possible to detect them if they are partially depolymerized, because depolymerized DNA is Feulgen negative.[136,158]

There are several possibilities in regard to the function of small lymphocytes in the cytoplasm of the columnar cells. In the first place, small lymphocytes may be wandering aimlessly and not have any functional significance, cause any metabolic disturbance to the columnar cell, or be altered structurally or physiologically. A second possibility is that the small lymphocyte enters the cytoplasm of the columnar cells, remains for a period during which it assimilates amino acids, nucleotides,[151] nucleosides,[62] and other substances essential for RNA synthesis, and then, as a plasmacyte, returns to the core of villi. However, plasmacytes are very rarely found within the columnar epithelium or adjacent to the basement membrane. The third possibility is that the small lymphocyte furnishes trephones to the columnar cell. Changes in DNA in the nuclei of lymphocytes in columnar epithelial cells have not been detected by use of the Feulgen method, nor has extrusion of DNA from nuclei of small lymphocytes.[93] Use of radioactive isotopes recently has demonstrated new facts that may explain the function of DNA in small lymphocytes in metabolism of columnar epithelia. Hewesy and Ottesen (1943) compared the daily renewal of DNA by use of P^{32} and found that 15 per cent of the DNA in the intestinal mucosa was renewed daily as compared with 5.7 per cent in the spleen, which was the second organ of turnover activity.[40] Studies with P^{32} have shown that lymphoid organs have a high turnover.[181]

The small rim of cytoplasm of small lymphocytes contains RNA (as indicated by use of RNAase and by staining with methylene blue in a solution at *p*H 4.9[72]) and could be a source of trephones that cannot be detected by histochemical methods because the cytoplasm of the small lymphocyte is surrounded by cytoplasm of the columnar cell. Use of the electron microscope, however, might indicate passage of substances such as granules from one cell to the other. The diffusion of RNA or its components from the cytoplasm of the small lymphocyte to the cytoplasm of the columnar cells would be very important in the metabolism of columnar cells. A high RNA content is characteristic of cells in which protein is being synthesized for either growth or secretion.[18,30,31] In addition, Binkley suggested that RNA is active in peptide synthesis.[40] Although most columnar cells of villi are not very active in secretion and do not divide, they might require a supplementary and extraneous source of RNA for assimilation.

Brachet[16] considers that "free" RNA (the RNA not combined with granules) occurs only in those cells that are in a state of active proliferation, and that the "free" fraction is very labile and "participates in the synthesis of thymonucleic acid and in the production of the coenzymes for phosphorylation." If the cytoplasm of small lymphocytes furnishes the free RNA for the columnar cell, the RNA could function as coenzymes for phosphorylation, because these columnar cells are most active in the phosphorylative processes. By use of radioactive methods, LeBlond and Stevens have calculated that mucosal cells survive for only 36 hours from the onset of mitosis in the crypt to extrusion into lumen.[171] The small rim of cytoplasm of small lymphocytes could be a source of adenylic acid for use by the columnar cells. In addition, the mobility of small lymphocytes into and out of the columnar cells also could be a mechanism for regulating riboadenylic acid in the cytoplasm of epithelium of villi.

WOUND HEALING

Plasmacytes develop from lymphocytes at sites of wound healing following an increase of protein in the tissue fluid. The amount and time during which plasmacytes develop from lymphocytes in these areas vary according to the amount of necrotic material, presence or absence of infection, size of wound, vascularity, and amount of plasma proteins. Thus, the original type of the wound (such as contused, thermal, chemical, lacerated, incised) and its later history (whether sutured plastically treated, open, cystic, infected, or the like) will determine whether it heals by "first intention" or by "granulation." Consequently, these factors may govern the extent and duration of exudation, the protein content of the exudate, the infiltration of lymphocytes, fibrinogenesis, and plasmacytogenesis.

The formation of plasmacytes in wound healing is a "bridge" mechanism whereby the increased protein of the first stage is retained locally for later use by proliferative cells. This bridging mechanism is essential because the initial irritant involved in inflammation, whether the substance provoked by it is histamine, the leukotaxin of Menkin,[129] or some other substance, has usually disappeared before the onset of mitotic proliferation in the tissues. Hyperemia is a prerequisite for increased function or growth; normally this increased circulation is controlled by hormones and neurones. However, in the case of healing of inflammatory tissues, the chemically induced increase in vascularity and the resultant increase in tissue fluid are characteristic of the first stage, while mitotic proliferation ushers in the last stage in the healing process.

The formation of plasmacytes from lymphocytes presents a form of local storage of RNA and promotes a resynthesis of the animal's own protein, from necrotic tissue and other sources, to some of the nucleic acid components utilized by proliferating cells, such as fibroblasts in the case of scar-tissue formation.

Many factors are related to the presence of lymphocytes and plasmacytes in wound healing. In most general descriptions of regeneration and wound healing, the presence of infiltrated lymphocytes and plasmacytes has been noted at certain stages, usually from 4 to about 12 days postoperatively,[120] by numerous investigators. However, this sequence does not obtain in some tissues as, for example, in nerve tissue. It is necessary to recognize that, as the process of wound healing proceeds, a number of conditions will be encountered that may inhibit or be subsidiary or supplemental to regeneration. Some of these more important factors are the amount of necrotic material, the presence or absence of ascorbic acid or infection, the extent and location of the wound, and the vascular relationships. It is interesting to note that numerous lymphocytes often persist in the lymphatics draining the site of the wound for some time after the injury apparently has healed.[120]

Fibrin plays an important part in the preparation of an area in tissues for the transformation of small lymphocytes into plasmacytes by causing incomplete stasis of venous blood, lymph and/or tissue fluid, and by forming a network of fibrin fibers with irregularly sized meshes, which helps confine protein and lymphocytes to the area containing the exudate or frank hemorrhage.[43]

Plasmacytogenesis occurs in the process of wound healing by granulation. In this method of wound healing, protein is locally increased in the tissue fluid by necrosis of cells, by hyperemia and the resulting increased capillary permeability, by formation of a fibrin network, and by varying degrees of lymph stasis. It appears that this kind of a situation is nearly ideal for the transformation of lymphocytes into plasmacytes.

The stages of wound healing indicate that plasmacytes have a function in storing proteins for use by regenerating cells. The development of plasmacytes from lymphocytes follows the localization of protein during the first stage of granulation tissue-formation and precedes active regeneration. The necrosis of tissue cells, dissolution of blood clots, exudation of proteins from blood capillaries, and proteolysis in polymorphonuclear leukocytes and monocytes increase the available proteins. This stage is followed by the development of some of the lymphocytes into plasmacytes.

Plasmacytes and lymphocytes, as well as other leukocytes, have been considered to have a function in resistance, chiefly by preventing infection of the exposed surface of granulating tissue.[170] However, lymphocytes and plasmacytes may have the primary purpose of localizing and synthesizing nucleic and amino acids and histones for use by the regenerating cells because they decrease as the areas are replaced by proliferation of fibroblasts or other cells and by deposition of collagen.

INFLAMMATION

Development of lymphocytes into plasmacytes in areas of stasis of extravascular fluids that have a high protein content also may account for the presence of both of these cells in subacute and chronic inflammation. This infiltration of inflammatory areas represents an intermediate process by which the proteins from exudate, or fluid containing products of necrotic tissue, cytolyzed polymorphonuclear leukocytes, or other blood cells are retained locally for later use by proliferative cells.

This process is basically the same at sites of chronic inflammation as in normal tissue and as in the core of intestinal villi, in which transformation of lymphocyte to plasmacyte occurs.[94, 118]

Lymphocytes and plasmacytes are usually numerous during the later stages of inflammatory processes. The first stages of inflammation are characterized by the injured tissue showing hyperemia and increased permeability of blood and lymph capillaries with infiltration of polymorphonuclear leukocytes as a result of the irritant, whether chemical, thermal, mechanical, or parasitic. Experimentally induced inflammations show that lymphocytes appear from blood and lymph vessels about the fourth day, become increasingly abundant, and are "most abundant after inflammation has lasted several days."[120] The earlier processes, particularly hyperemia and increased capillary permeability, commonly are responsible for the increased amount of protein in the tissue fluid in the region of inflammation. However, the amount of protein is increased locally during the acute stage of inflammation by its increased passage through the more permeable capillaries and as a result of proteolysis of injured and necrotic tissue, dead polymorphonuclear leukocytes and, in some cases, of cytolyzed erythrocytes. This increase in protein usually persists in subacute and chronic inflammation and in normal wound healing for a period sufficiently prolonged to permit infiltration of lymphocytes and the transformation of some of them into plasmacytes.

Formation of plasmacytes does not occur in all lesions that contain infiltrations of lymphocytes. Some chronic inflammatory infiltrations may be composed almost entirely of lymphocytes, whereas most of the cells in several other conditions are plasmacytes. A high proportion of plasmacytes has been shown in primary syphilitic lesions as compared with tubercular lesions,[143] while in paresis infiltrated plasmacytes are much more numerous than in syphilitic lesions of the brain.[135] However, this difference in number apparently is related to the extent and duration of bionecrosis in the brain rather than to the pathological entity as such.

Edema

Edema fluid, by definition, is intercellular or tissue fluid, but it is pathological in origin since it is formed only under abnormal or disturbed physiological conditions of local vessels or of the circulatory system. When the amount of tissue fluid, which is essentially a filtrate from the blood capillaries, becomes sufficiently great to produce swelling, the condition is designated "edema" and, the contained fluid is therefore known as edema fluid.[45] Exudative edema fluid, or exudate, characteristically has a high specific gravity, and contains white and sometimes red blood cells and an unusual amount of plasma protein, including fibrin, which has escaped from blood vessels as a result of inflammatory processes prevailing in the tissues through which the blood vessels pass. Transudates, such as occur in cardiac or renal edema, characteristically have a comparatively low specific gravity, contain less fibrin and other proteins than any other edema fluid,[14,126] and occur under noninflammatory conditions with leaking capillaries; however, they are not favorable for plasmacytogenesis. It is thus apparent that the content of lymphocytes and plasmacytes in edema fluid is more closely related to the protein content and duration than to the volume or extent of the edematous involvement.

Celsus (25 B.C. to 45 A.D.) recognized in the involved tissues in true inflammation the changes that he described under the four cardinal symptoms of inflammation.[116] Incidentally, his terminology still is employed. A century ago, Cohnheim, Samuel and Virchow observed that inflammatory processes produce physiological changes in the local circulation.[116] A diffusible component that has growth-promoting properties has been considered to be present in inflammatory exudates.[131] Samuel (1873) believed that changes in the walls of these blood vessels gave origin to the exudate that forms the fluid in exudative edema.[130] However, Cohnheim (1866 and later), was responsible for paving the way for further experimental interpretation of inflammatory processes[130] and edematous relationships. Edema supervenes if this steady state is interrupted by osmotic, mechanical, and/or inflammatory changes, so that more fluid enters than leaves the intercellular spaces. Whether the edema fluid will be favorable for the transformation of small lymphocytes into plasmacytes may depend upon its protein content. In turn, the protein content is related most commonly to conditions favoring passage of the larger protein molecules through the capillary wall into a situation where it is detained, generally by formation of a fibrin network.

Most investigators agree that in the higher animals inflammation involves local tissues and complex blood-vascular and lymphatic reactions,[42] while Ehrich[57] holds that this process begins with changes in the ground substance, and he points out that Rössle believed that "vascular reaction is not an essential part of inflammation." Dougherty (1953) stresses the response of interstitial connective tissue and supports his tenet by citing the belief that muscle cells and capillaries do not become inflamed.[86] The foregoing discussion indicates that an incisive definition of inflammation is not available.

Irrespective of the various factors incident to inflammatory processes, it is reasonably certain that excape of an appreciable amount of protein with the exudate occurs only in the presence of increased capillary permeability and, furthermore, transformation of an appreciable percentage of the aggregated lymphocytes into plasmacytes in inflammatory lesions is dependent primarily upon an increase in amount of protein in the exudate over that in the normal tissue fluid. Thus, by causing capillary leakage, histamine may well be one of the potent agents within the body having the function of aiding or even indirectly controlling the formation of plasmacytes in subchronic and chronic inflammation, so that the RNA content of these cells may play an essential part in wound healing.

LYMPHOCYTES AND PLASMACYTES IN TUMOR GROWTH

Lymphocytes and plasmacytes are sometimes mentioned in descriptions of the cellular infiltrations that occur around the margins and within the stroma of some tumors. The significance of lymphocytic concentration has been a controversial subject because infiltrations of lymphocytes and plasmacytes are characteristic of many preneoplastic lesions and because these cells often occur at the margins of spontaneous tumors and are often present around progressive as well as regressive implants of tumors.

The cause of infiltration of lymphocytes around regressing tumor implants and their transformation into plasmacytes is thought to be the same as in wound healing and subchronic and chronic inflammation — the presence of hyperemia and increased permeability of capillaries with exudation of excess protein into the tissue fluid. Usually the products of bionecrosis also are available. However, the apparent difference in function of lymphocytes and plasmacytes around progressing and regressing tumor implants is attributed to the particular type of cell that utilizes the nucleic and amino acids. In an established tumor the neoplastic cells use the nucleoprotein and other substances in tissue fluids, whereas, in a regressing tumor, such as a heterologous transplant, the proliferating cells are the fibroblasts of the host and, consequently, the growth-promoting substances are used by these fibroblasts, rather than by the heterologous tumor cells.

Failure to consider the fact that lymphocytes stimulate growth of normal cells resulted in the confusion in interpreting the relation of lymphocytes to tumor growth. On the one hand, observations on infiltration of lymphocytes in preneoplastic lesions, in the connective tissue cultures, indicate that lymphocytes increase tumor growth. On the other hand, studies on regression of tumor implants and comparison of the percentage of "takes" in various organs, such as the brain, testis, and connective tissue, and in chick embryos were interpreted as indicating that lymphocytes have a function in resistance to cancer.

The literature on the relation of lymphocytes to cellular proliferation was summarized in 1901 by Levin,[107] who states that the theory that some external agent may incite proliferation of various cells was first advanced by Virchow. Klebs *et al.*[107] believed that cellular proliferation resulted from conjugation between a tissue cell and a leukocyte or that, before dividing, the cells underwent a certain transformation. The idea that conjugation between a cell and a leukocyte is the cause of cancer has not, of course, been established. However, the nutrient and energy relations of DNA and RNA synthesis and the storage of these substances by lymphocytes and plasmacytes for use by other cells represent a broader development of this idea.

About the time that Murphy[140] and his co-workers were developing their studies on the relationship of lymphocytes in resistance to tumor growth, Carrel[27] was investigating the growth-promoting effects of lymphocytes. In 1923 Carrel and Ebeling[29] noted that lymphocytes not only lived but proliferated in a medium containing blood serum. Fibroblasts, however, were unable to survive in this medium without the addition of lymphocytes and large mononuclear cells. Carrel[28] expressed the belief that fibroblasts do not find in serum all the essential substances that are required for synthesis of protoplasm and must, therefore, receive material from other sources. In 1933 Dustin[56] held that lymphocytes have an important function in the metabolism of nucleoproteins. Jordan and Speidel[92] also noted the growth-promoting function of leukocytes and suggested that lymphocytes may be an embryonic relic that persists and stimulates regions where stimulation is needed.

Infiltrations of lymphocytes and plasmacytes at areas of carcinogenesis and at sites of invasion by tumor may have a function in synthesizing and storing nucleoprotein. Although the invading margins of some spontaneous and implanted tumors usually contain infiltrations of lymphocytes and plasmacytes, other areas invaded by the same tumor may not contain infiltrations of these cells. Woolley[180] states that the reports of Sittenfield and Stevenson questioned Murphy's[140] tenet that lymphocytes are important in resistance to cancer and tuberculosis. Murphy[140] reported that tumor implants grew well in embryos before the appearance of lymphoid cells; however, Stevenson[180] concluded that the presence of adult chicken spleen did not inhibit growth of any tumors in the chick embryos. Woolley[180] attributed the differences in results obtained by these two investigators to the methods of handling tissues, time relations, and other factors, and concluded that the lymphocytic reaction in cancers is totally extraneous as far as resistance is concerned.

Infiltration of lymphocytes and the formation of plasmacytes from some of the lymphocytes around heterologous tumor transplants, as in mouse tumors transplanted in hamsters,[97] is considered to be due to increased protein resulting from capillary leakage and the disintegration of heterologous and other cells. These plasmacytes are a source of increased RNA and gamma-globulins, as well as of antibodies, and their disintegration releases trephones that can be used for growth of fibroblasts in reactive inflammation around foreign transplants and that thereby increase fibroblastogenesis around heterotransplants.

If the basic function of lymphocytes is to synthesize and store nucleoproteins, it would be difficult to explain how these cells could promote growth in one tissue and inhibit it in another, unless some differential barrier, such as heterogenicity, is operative. An essential point to be considered here is that the heterologous tumor cells provoke a foreign protein reaction, and that these tumor cells are unable to utilize the nucleic acids present in the infiltration lymphocytes. However, this situation is usually accompanied by fibroblastic proliferation, with the result that destruction of the heterologous transplant would be chiefly the result of fibrocytogenesis, a process that is accelerated by trephones from lymphocytes and plasmacytes.

The idea that lymphocytes are primarily antagonistic to cancer growth is inconsistent with the well-known fact that lymph nodes are generally the most susceptible, or at least one of the most frequently involved, of all structures to metastasis of both carcinoma and sarcoma.[33,176,177] This discrepancy was explained by invoking the possibility that the lymphocyte is impotent while within the lymph node, but acquires functional activity in resistance to cancer after it leaves the lymph node and reaches the blood.[140]

The idea that leukocytes, especially the lymphocytes, furnish nutrients for growth of cells was supported by Virchow and other investigators during the latter half of the Nineteenth Century until this idea was eclipsed by the work of Murphy[140] and his colleagues on the viability of heterologous tumor transplants. The fact that the work of these investigators indicated that lymphocytes resist, rather than promote, the growth of heterologous tumor transplants, presumably by direct effect upon the implant, gave rise to the widespread acceptance of the opinion that lymphocytes inhibit tumor growth.

This involves a number of variables that cannot justly be evaluated in interpreting the reaction of lymphocytes to implants of tumor. Perhaps the most obvious is the "degree of foreignness of the protein."[114] The definition of autotransplants as tissue from the same individual rules out the "foreign protein reaction," but there remain variations in vascularity and capillary permeability and other differences in the new site of implantation, such as permeability of the connective tissue ground substance. The infiltration of lymphocytes around autotransplants is usually less than that occurring around isotransplants,[114] and much less than that occurring around heterotransplants.[97]

Heteroplastic implantation unquestionably introduces foreign protein, but implants of homeotransplants also vary in their "degree of foreignness,"[114] and for this reason they do not provide a reliable basis for interpretation of lymphocytic reaction. Transplantation of tissue, even between individuals of the same inbred strain, introduces the possibility of numerous differences in the foreign protein reaction; for example, antibodies to *Salmonella* may be present in a donor and absent in a host. In evaluating the immunity response following implantation of a tumor from one inbred line to another inbred line, it is important to consider that infiltrations of lymphocytes and plasmacytes around the implant and systemic changes in lymphoid organs could be due to differences in structures of globulins or antibodies in addition to the implanted tumor tissue. A bacterial or viral infection also alters the vascular response around the tumor implant and thereby, in addition to inducing a systemic reaction, alters the speed with which a tumor regresses or grows.

Another variable is the difference in latent period required to establish the implant, for it is known that the duration of time required to establish vascularity of grafts varies. Tyzzer[169] states that a blood supply was available for tumor from waltzing mice implanted into common mice at 6 days. Boyd[13] observed the formation of new capillaries in the implant as early as 24 hours after implantation of breast carcinoma of mice into other mice. Merwin and Hill[132] report that some homeografts are vascularized during the third to fifth week after transplantation, whereas others are vascularized within a week, and that the nonvascularized homeografts survived longer. Part of these differences in time required for vascularization of grafts in transplanted tumors may be due to failure to recognize differences in implantation sites; the subcutaneous site, for example, is much less vascular than the subpannicular site. Knight[99] holds that thin-walled capillaries are necessary for the growth of skin graft in order to permit passage of growth-stimulating products, and that the type of capillary is of great importance to "takes" of grafts.

Significance of Infiltration

The significance of the infiltration of lymphocytes around homeotransplants and heterotransplants has been considered from numerous viewpoints. As early as 1915, Tyzzer[169] stated that the literature on tumor immunity was so extensive it could not be discussed in a single article. Infiltration of lymphocytes around syngenesiotransplants, homeotransplants, and heterotransplants has been considered as an immunity reaction.[113] Loeb[112] recognized that transplantation of experimental tumors represents the introduction of foreign cells, and he stated that "the body does not react defensively against spontaneous tumors." Meyers and Hesselberg also observed that homeotransplants of normal tissues were surrounded by lymphocytes and by proliferation of fibroblasts that "strangulate the foreign cells."[113] Murphy[139] states that "lymphoid tissue is an important factor in the immunity reaction to transplanted cancers of mice." He notes also that immune mice show indications of lymphoid hyperplasia, and that lymphoid depletion is generally present in susceptible mice.

The infiltration by lymphocytes has usually, but not without question, been considered a cause of necrosis of cancer cells. Myers and Hesselberg hold that the heterotransplants of normal tissues died as a result of attack by lymphocytes and by proliferation of fibroblasts that strangled the foreign cells.[113] Loeb[113] held that tumor immunity was the result of an inflammatory reaction, while Tyzzer[169] also considered that the so-called immunity reaction around a tumor transplanted from Japanese waltzing mice into common mice was due to an inflammatory reaction provoked by foreign protein.

Recently, Favour[67] stated that the destructive process in homeografts is coincident with the appearance of lymphocytes at the site of the transplant, and that the lymphocytes *in vitro* are the "trigger mechanism for initial limited cell destruction of polymorphonuclear cells." Ewing[65] holds that the infiltration of lymphocytes and plasmacytes in spontaneous growth of malignant tumors and the inclosure of islands of human mammary tumor cells by masses of infiltrating lymphocytes must be regarded as a defense process against the tumor. However, Murphy[140] was the most ardent of the advocates who held that lymphocytic infiltration of tumor sites is a defense process against growth of the tumor, although he stated later[141] that research on natural, acquired, or induced resistance to injected cells had not only failed to throw light on the mechanism in the body for resisting the disease, but had not contributed to any understanding of these processes that would be expected to lead to improved chemical methods of treatment. Murphy[141] further indicates that, with regard to heterotransplantation, the body's natural resistance "is not resistance against cancer as a disease but against the introduced cells of another individual of a different genetic make-up."

The infiltration by lymphocytes could be explained just as well by considering it as a response to the presence of necrotic tissue instead of as the cause of the necrosis. This explanation is in agreement with the known facts of infiltration of lymphocytes at sites of subchronic or chronic inflammation and wound healing, because in these processes necrosis of tissue and infiltration of some polymorphonuclear leukocytes precede the infiltration of lymphocytes. Furthermore, the presence of lymphocytes and plasmacytes in most normal tissues and organs is related to protein storage. Infiltration often follows, but an increase in lymphocytes and plasmacytes rarely precedes necrosis of tissue. The population of lymphocytes in noncancerous lymph nodes decreased in animals having a growing tumor,[140] an effect not inconsistent with the hypothesis that lymphocytes are protein storage cells and are caused by, or related to, onset of inanition in the host. Lymphocytes that have infiltrated homeografts and become plasmacytes also have been considered an effect of the immune reaction rather than the cause.[39] The disintegration of plasmacytes has been attributed to vascular failure rather than to hormonal mechanisms.[39]

The infiltration of lymphocytes and plasmacytes around a regressive homeotransplant or heterotransplant represents two possibilities: (1) the storage of the host's own protein at a localized site, as in areas of edema and other conditions resulting from increased capillary permeability or (2) cytolysis and the production of antibodies against the homeotransplant and heterotransplant by plasmacytes. Several proteolytic enzymes occur in lymphocytes and plasmacytes and may act upon foreign tumor implants, but these same enzymes also occur in numerous other cells. Favour[67] considers that lymphocytes and plasmacytes that occur at sites of graft rejection may produce antibodies *in situ,* although he notes also that antibodies are probably not as readily detected in circulation. However, antibodies can elicit localized anaphylactic responses of the type occurring in polyarteritis, or the Arthus phenomenon. Plasmacytes at sites of degenerating cancer grafts may not only produce antibodies, but also may have the same function as in wound healing and chronic inflammation; that is, they function in "bridging" the gap between the period of acute inflammation and the onset of proliferation of fibroblasts or other cells.

Conditioning Hosts

Several of the more commonly used methods of host conditioning significantly deplete the circulating lymphocytes and the lymphoid tissues, but this effect has been considered by comparatively few investigators. Parenterally administered cortisone normally decreases the rate of growth of homeotransplanted tumors[35,83] and retards proliferation of capillaries, elaboration of reticulum, growth of fibroblasts around the implant, and vascularization of the implant.[83] Human tumors implanted in rats grew in animals receiving cortisone and X irradiation and in those receiving cortisone alone, but not in controls.[166] Regression of human tumors in rats, however, was reported to occur 19 to 20 days after transplantation and was found to be concomitant with a decrease in lymphocytes in the peripheral blood.[81] The inflammatory reaction around human malignant tumors transplanted to the anterior chamber of the eye was decreased in guinea pigs that received cortisone.[167] Injections of cortisone into hamsters also decreased the inflammatory response around heterologous tissue implanted into the cheek pouches.[117] Baserga and Shubik[3] report an increased incidence of metastasis of transplanted tumors in cortisone-treated animals; but this treatment did not influence growth of a heterologous transplant of adenocarcinoma from DNA mice to Swiss mice. These investigators think that caloric restriction probably would not be the cause of the increase in metastasis, as Tannenbaum and Silverstone (1953) observed in mice. These relations, however, might be due to the fact that cortisone increases protein catabolism and decreases the rate of tumor growth, thereby increasing the life span of the tumor for a time sufficient to permit formation of macroscopic metastases because the incidence of metastasis is correlated with the duration of malignant tumors.[33,34]

Stoerk[161] states that small lymphocytes are more readily suppressed by cortisone than other mononuclear leukocytes, and that autologous lymphoid cells failed to produce resistance to cancer grafts, but that heterologous or injured homologous cells did so. The injection of cortisone increased the distribution of miliary necrosis within a pleomorphic cell sarcoma (HS-6) in hamsters.[35,97] Therefore, in proper dosage, cortisone might be expected to increase the percentage of metastases.

SUMMARY

(1) The purpose of this monograph is to present a working hypothesis showing that the function of the small lymphocyte is to synthesize, store, and transport nucleoproteins for use by other cells, and that the function of the plasmacyte is to synthesize and store RNA and gammaglobulins, instead of having only the delimited function of producing antibodies.

(2) The size, motility, mobility, ubiquitous distribution, absence of mitosis, high nucleocytoplasmic ratio, and rapid turnover are characteristics that enable small lymphocytes to function as trephocytes. Small lymphocytes within columnar epithelial cells of intestinal villi are considered intracellular trephocytes, rather than cells in the process of elimination.

(3) Plasmacytes, which likewise do not divide, develop from lymphocytes in normal animals in the lymph nodes, spleen, hemolymph nodes, and tunica propria of the intestine. These organs and tissues are characterized by partial stasis of fluid containing abnormally large amounts of plasma proteins.

(4) The lymph nodes store nucleoproteins between tissue fluid and blood stream, whereas the spleen, and possibly hemolymph nodes, regulate and store protein within the blood vascular system. Peyer's patches, solitary follicles, the vermiform appendix of rabbits, and small

lymphocytes and plasmacytes in the other regions of the intestinal mucosa synthesize and store amino acids and nucleoproteins between the lumen of the intestine and the blood and lymph.

(5) Lysis of small lymphocytes and plasmacytes in the hepatic portal vein, spleen, mesenteric lymph nodes, mucosa of the intestine, and in other portal organs may be related to protein synthesis in the liver.

(6) Infiltrations of small lymphocytes and plasmacytes in areas of wound healing and chronic inflammation concentrate proteins for use by other cells in regenerating or replacement processes.

(7) Infiltrations of these cells indirectly antagonize growth of heterologous tumor transplants by stimulating fibrocytogenesis in the host.

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